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# Biochemical Changes in Liver and Blood During Liver Fattening in Rats

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**Summary:** Excessive fat accumulation in the liver is a common metabolic disorder seen in humans and animals. Fatty liver was induced in the rat by feeding the animals with a sucrose rich diet containing 1% orotic acid for 2–3 weeks.

In the sera from fatty liver rats there were significant changes in the level of alanine aminotransferase (+ 68.7%), malic dehydrogenase (+ 77.8%), γ-glutamyl transpeptidase (−53.4%) and total lipids (+ 26.6%). There were small to no changes in the levels of aspartate aminotransferase, glucose-6-phosphate dehydrogenase, lactic dehydrogenase, aldolase, malic enzyme, 6-phosphogluconic acid dehydrogenase, alkaline phosphatase and albumin.

In fatty liver, significant differences were seen in the levels of glucose 6-phosphate dehydrogenase (+ 235%), malic enzyme (+ 170%), γ-glutamyl transpeptidase (+ 113%), 6-phosphogluconate dehydrogenase (+ 63%), aspartate aminotransferase (+ 35.6%), malic dehydrogenase (+ 38%), lactic dehydrogenase (+ 37%), and alanine aminotransferase (− 23%).

Comparison of the non-fatty part with the fatty part of the fatty liver showed larger changes in the non-fatty part of the liver, suggesting that during the fattening process, there is an induction of enzymes in the liver reaching a peak prior to lipid accumulation, declining thereafter during liver fattening. The increase in NADPH-generating lipogenic enzymes suggests that accumulated fat in the liver is at least partially from de-novo increased synthesis in the liver.

*Änderungen biochemischer Kenngrößen in Leber und Blut während Leberverfettung bei der Ratte*

**Zusammenfassung:** Die exzessive Anhäufung von Fett in der Leber ist eine bei Mensch und Tier beobachtete Stoffwechselstörung. Durch Verfütterung einer 1% Orotsäure enthaltenden Saccharose-reichen Diät für 2–3 Wochen wurde bei Ratten eine Fettleber hervorgerufen.

Im Serum der Ratten mit Fettleber waren signifikant verändert: Alaninaminotransferase (+ 68,7%), Malatdehydrogenase (+ 77,8%), γ-Glutamyltransferase (−53,6%) und Gesamt-Lipide (+ 26,6%); gering oder nicht verändert waren: Aspartataminotransferase, Glucose-6-phosphatdehydrogenase, Lactatdehydrogenase, Aldolase, Malat-Enzym, 6-Phosphogluconatdehydrogenase, Alkalische Phosphatase und Albumin.

In der Fettleber waren signifikant verändert: Glucose-6-phosphatdehydrogenase (+ 235%), Malat-Enzym (+ 170%), γ-Glutamyltransferase (+ 113%), 6-Phosphogluconatdehydrogenase (+ 63%), Aspartataminotransferase (+ 35,6%), Malatdehydrogenase (+ 38%), Lactatdehydrogenase (+ 37%) and Alaninaminotransferase (−23%).

Der Vergleich nicht-verfetteter und verfetteter Teile der Fettleber zeigte stärkere Veränderungen in nicht-verfetteten Teilen. Dies weist darauf hin, daß während des Verfettungsvorgangs in der Leber Enzyme induziert werden, deren katalytische Aktivität vor der Fettakkumulation am höchsten ist und während des Verfettungsvorgangs wieder abfällt. Der Anstieg der für die Lipogenese bedeutsamen NADPH-generierenden Enzyme deutet darauf hin, daß in der Leber akkumuliertes Fett zumindest teilweise aus gesteigerter Neusynthese in der Leber stammt.

## Introduction

Steatosis is a common and non-specific response of the liver to different forms of acquired injury or inherited metabolic derangement. Knowledge of the biochemical changes leading to the excessive fat accumulation is fragmentary only. Because of the wide diversity of functions and tremendous regenerative and reserve capacity of the liver, the detection of fatty liver is difficult before serious impairment of hepatic function or cellular damage occurs.

Because of the interplay of the various pathological mechanisms by which fat is accumulated in the liver, it is hard to make a precise statement on the mechanisms which operate in any given case. It is however, possible to group the pathological mechanism into

(1) those which involve an imbalance of nutritional or metabolic factors such as starvation and a diet low in proteins or high in fat or carbohydrates, and endogenous imbalances especially those involving hormones

(2) those due to toxins, chemical poisons etc.

(3) those which are a result of anoxia, (1 – 5).

The biochemical changes in the blood and liver of geese during force feeding showed significant changes in liver and blood enzymes, indicating that induction of many enzymes in the liver had taken place in parallel with the fat accumulation in the liver. At a stage of massive liver enlargement, cellular damage occurred and liver enzymes were released to the blood (5). To evaluate whether these changes also occur in another animal model, rats fed on a rich carbohydrate diet were investigated. the present study describes the changes which take place in the rat liver during liver fattening.

## Materials and Methods

### Animals

Male Sprague Dawley rats weighing 300 – 350 g were used. Fatty liver was induced by feeding the animals with a sucrose-rich diet (consisting of 69.8% sucrose, 20% vitamin-free casein, 4% salt mixture and 2.2% vitamin mixture) given for 2 – 3 weeks, followed by the same diet containing 1% orotic acid for 3 – 5 weeks. The controls received a commercial chow diet (7).

The animals were sacrificed following anaesthesia with ether and blood was collected from the abdominal aorta. The blood was allowed to clot at room temperature and serum was obtained.

### Liver

The liver was removed, blotted and weighed. Two separate experiments were conducted:
1) using the entire liver;
2) separating the fatty liver into fatty and non-fatty parts.

Liver was cut into small pieces, washed three times with buffer (Tris-HCl 0.02 mol/l, pH 7.4) and homogenized with a *Potter-Elvehjem* glass teflon homogenizer in 10 volumes (w/v) of the same buffer. The homogenate was centrifuged at 10000 *g* for 30 min; the supernatant was removed and recentrifuged at 100000 *g* for 60 min in a MSE-50 ultracentrifuge, and the supernatant was used for enzyme studies (5).

The liver was separated into fatty and non-fatty parts according to colour. The degree of fatty infiltration was determined microscopically following fixation and staining.

### Enzymes

The following enzymes were measured in the supernatant obtained and sera.

*L*-lactate: NAD oxidoreductase EC 1.1.1.27, *L*-malate: NAD oxidoreductase EC 1.1.1.37, *L*-isocitrate: NADP oxidoreductase decarboxylating EC 1.1.1.42, *D*-glucose 6-phosphate: NADP oxidoreductase EC 1.1.1.49, 6-phospho-*D*-gluconate: NADP 2-oxidoreductase decarboxylating EC 1.1.1.44, *L*-malate: NADP oxidoreductase oxaloacetate decarboxylating EC 1.1.1.40, orthophosphoric monoester phosphohydrolase, alkaline optimum EC 3.1.3.1, orthophosphoric monoester phosphohydrolase acid optimum EC 3.1.3.2, *L*-aspartate 2-oxoglutarate aminotransferase EC 2.6.1.1, *L*-alanine 2-oxoglutarate aminotransferase EC 2.6.1.2, *L*-iditol: NAD 5-oxidoreductase EC 1.1.1.14, 5-glutamyl peptide: amino acid 5-glutamyltransferase EC 2.3.2.2 and *D*-fructose 1.6-diphosphate: *D*-glyceraldehyde 3-phosphate lyase EC 4.1.2.13.

Serum enzymes were determined spectrophotometrically according to conditions described elsewhere (8). Whenever enzymes were determined in the liver, aliquots from the supernatant were taken and measured under the same conditions (8). Appropriate amounts were taken to give activities within the optimal range. Whenever the activities were too high, the samples were diluted with the homogenizing buffer solution. Enzyme activities were calculated per mg protein in the enzyme containing sample.

### Metabolites

In addition, albumin and total lipid were measured in the serum. Albumin was determined according to *Bartholomew & Delaney* (9), total lipids with the phosphovanilin method according to *Frings* et al. (10) and protein according to Lowry et al. (11).

### Statistics

Mean and standard deviation (S. D.) and standard error of the mean (SEM) were calculated and the t-test was used to determine levels of significance.

## Results

The weights of livers from control and fatty liver-induced rats are shown in table 1. Fatty liver was larger by 18.9% than the control group. Liver to body ratio was also increased by the same magnitude. There was a significant decrease in the protein content of the fatty liver as evaluated by mg protein/g fresh tissue. The change seen although negative was with a similar order of magnitude (tab. 1). While there was no change in the concentration of serum albumin, there was a significant increase (26.6%, $p < 0.01$) in the serum lipids from the rats with fatty liver (tab. 1).

Levels of enzymes in the sera of controls and fatty liver-induced rats are given in table 2. Significant increases in the activities of alanine aminotransferase (68.7%, $p < 0.01$) and malic dehydrogenase (77.8%, $p < 0.01$) were seen in the sera of rats with fatty liver. Although not significant, the levels of aspartate aminotransferase, aldolase, glucose 6-phosphate dehydrogenase and lactic dehydrogenase were higher also. A significant decrease (53.4%, $p < 0.01$) in the serum level of γ-glutamyl transpeptidase was seen, while no change was seen in the levels of alkaline phosphatase, malic enzyme and glucose 6-phosphate dehydrogenase (tab. 2).

Levels of enzymes in normal and fatty liver are shown in Table 3. The enzyme showing the largest increase was glucose 6-phosphate dehydrogenase which changed from the mean level of 17.2 U/g protein in the controls, to 57.6 U/g protein in the fatty liver rats, an increase of 235% ($p < 0.01$). The activity of malic enzyme, increased significantly ($p < 0.01$) from 10.3 to 27.8 U/g protein in the fatty liver (an increase of 170%).

The enzymes γ-glutamyl transpeptidase, aldolase, 6-phosphogluconate dehydrogenase, malic dehydrogenase, lactic dehydrogenase and aspartate aminotransferase increased significantly by 113, 95, 63, 38, 37 and 36 percent, respectively. The levels of sorbitol dehydrogenase and alanine aminotransferase were significantly lower ($p < 0.05$) in the fatty liver. In the fatty liver, small insignificant changes were seen for the enzymes alkaline phosphatase, acid phosphatase and isocitric dehydrogenase (tab. 3).

The distribution of enzyme activities in the non-fatty and fatty parts of the fatty liver vs. the non-fatty liver control are depicted in table 4. Whenever a change was seen in the fatty liver, it was greater in the non-fatty part than in the fatty part.

The activity of glucose 6-phosphate dehydrogenase in the non-fatty control liver was 12.7 ± 2.1 U/g protein, 52.7 ± 8.0 U/g protein in the non-fatty part of the fatty liver (an increase of 315%) and 27.0 ± 5.0 U/g protein in the fatty part of the fatty liver (an increase of 113%) (tab. 4). Whenever the entire fatty liver was compared with the control, this increase was 235% (tab. 3). A similar picture was seen with malic enzyme, which showed values of 9.8 ± 0.6, 22.6 ± 2.6 and 16.2 ± 1.7 U/g protein in the non-fatty liver control, the non-fatty part and the fatty part of the fatty liver respectively, with increase of 131% and 65% (tab. 4). This pattern was consistent also with the enzymes glucose 6-phosphate dehydrogenase, malic dehydrogenase and lactic dehydrogenase.

A similar pattern, but in the opposite sense, was seen with the enzyme sorbitol dehydrogenase with decreased levels of 35% and 21% in the non-fatty and fatty parts respectively. An exception to this pattern was seen with the enzyme aldolase, which showed larger changes in the fatty part than the non-fatty part (tab. 4).

Tab. 1. Liver weight, serum albumin and total lipids from normal and fatty-liver rats.

| | Statistics | Liver weight (g) | Protein/tissue (mg/g) | $\frac{\text{Liver}}{\text{Body}} \times 100$ | Albumin (g/l) | Total lipids (g/l) |
|---|---|---|---|---|---|---|
| Control | $\bar{x}$ | 15.99 | 57.0 | 4.30 | 34.40 | 2.82 |
| | S.D. | 1.15 | 9.4 | 0.30 | 1.30 | 0.36 |
| | S.E.M. | 0.37 | 1.7 | 0.08 | 0.40 | 0.09 |
| | n | 17 | 17 | 17 | 12 | 15 |
| Fatty liver | $\bar{x}$ | 19.02*) | 46.8*) | 5.13*) | 34.40 | 3.57*) |
| | S.D. | 3.52 | 6.6 | 0.80 | 1.20 | 0.96 |
| | S.E.M. | 0.75 | 1.1 | 0.17 | 0.30 | 0.21 |
| | n | 22 | 22 | 22 | 17 | 22 |
| Change from control (%) | | + 18.9 | −17.9% | + 19.3 | n. c. | + 26.6 |

*) $P < 0.01$

Tab. 2. Levels of enzymes in sera (U/l) from normal and fatty-liver rats.

| | Statistics | Aspartate amino-transferase | Alanine amino-transferase | Alkaline phos-phatase | Malic enzyme | Glucose 6-phosphate dehydro-genase | 6-Phospho-gluconate dehydro-genase | Isocitric dehydro-genase | Malic dehydro-genase | Lactic dehydro-genase | Aldolase | γ-Glutamyl-trans-peptidase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | $\bar{x}$ | 50.9 | 8.11 | 82.7 | 12.46 | 2.77 | 12.55 | 5.43 | 315 | 824 | 48.8 | 17.4 |
| | S.D. | 11.6 | 2.35 | 33.9 | 4.98 | 1.83 | 2.67 | 4.23 | 150 | 242 | 4.3 | 1.3 |
| | S.E.M. | 2.8 | 0.63 | 8.2 | 1.20 | 0.61 | 0.95 | 1.13 | 36 | 59 | 2.1 | 0.6 |
| | n | 17 | 14 | 17 | 17 | 9 | 8 | 14 | 17 | 17 | 4 | 4 |
| Fatty liver | $\bar{x}$ | 60.4 | 13.68*) | 84.7 | 12.37 | 2.76 | 14.21 | 4.76 | 560*) | 905 | 59.7 | 8.1*) |
| | S.D. | 15.4 | 10.06 | 44.1 | 4.32 | 2.90 | 4.42 | 2.86 | 373 | 308 | 5.2 | 3.0 |
| | S.E.M. | 3.3 | 2.15 | 9.4 | 0.97 | 0.84 | 1.33 | 0.74 | 79 | 66 | 2.0 | 1.5 |
| | n | 22 | 22 | 22 | 20 | 12 | 11 | 15 | 22 | 22 | 7 | 4 |
| Change from control (%) | | + 18.7 | + 68.7 | n. c. | n. c. | n. c. | + 13.9 | −12.3 | + 77.8 | + 9.8 | + 22.3 | −53.4 |

*) $P < 0.01$

Tab. 3. Enzyme levels in livers (U/g protein) from normal and fatty-liver rats.

| | Statistics | Aspartate amino-transferase | Alanine amino-transferase | Alkaline phos-phatase | Acid phos-phatase | Malic enzyme | Glucose 6-phos-phate dehydro-genase | 6-Phospho-gluconate dehydro-genase | Isocitric dehydro-genase | Malic dehydro-genase | Lactic dehydro-genase | Aldolase | Sorbitol dehydro-genase | γ-Glutamyl trans-peptidase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | $\bar{x}$ | 298 | 162 | 0.80 | 46.2 | 10.3 | 17.2 | 26.5 | 151 | 2468 | 1480 | 35.5 | 94.9 | 1.36 |
| | S.D. | 61 | 30 | 0.17 | 4.9 | 5.8 | 11.3 | 8.5 | 22 | 539 | 361 | 6.7 | 22.3 | 0.52 |
| | S.E.M. | 17 | 8 | 0.05 | 1.7 | 1.4 | 2.3 | 2.8 | 5 | 128 | 87 | 3.4 | 5.4 | 0.23 |
| | n | 13 | 13 | 12 | 8 | 17 | 16 | 9 | 17 | 17 | 17 | 4 | 17 | 5 |
| Fatty liver | $\bar{x}$ | 404*) | 125*) | 0.72 | 42.0 | 27.8**) | 57.6**) | 43.1**) | 165 | 3405**) | 2033**) | 69.1**) | 72.5*) | 2.89*) |
| | S.D. | 99 | 45 | 0.30 | 5.5 | 11.7 | 28.4 | 15.8 | 88 | 913 | 444 | 37.5 | 16.4 | 1.29 |
| | S.E.M. | 26 | 12 | 0.07 | 1.7 | 2.3 | 6.2 | 4.0 | 17 | 179 | 89 | 11.3 | 3.2 | 0.58 |
| | n | 14 | 14 | 21 | 10 | 25 | 21 | 16 | 26 | 26 | 25 | 11 | 26 | 5 |
| Change from control (%) | | + 36 | − 23 | −10 | − 9 | + 170 | + 235 | + 63 | + 9 | + 38 | + 37 | + 95 | −24 | + 113 |

*) $P < 0.05$ **) $P < 0.01$

Tab. 4. Enzyme levels in control and parts of fatty-liver (U/g protein) from rats.

| | Statistics | Aspartate amino-transferase | Alanine amino-transferase | Aldolase | Alkaline phosphatase | Malic enzyme | Isocitric dehydro-genase | Glucose 6-phosphate dehydro-genase | 6-Phospho-gluconate dehydro-genase | Malic dehydro-genase | Lactic dehydro-genase | Sorbitol dehydro-genase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | $\bar{x}$ | 246 | 129 | 35.5 | 0.60 | 9.8 | 135 | 12.7 | 35.0 | 2937 | 1459 | 91 |
| | S.E.M. | 34 | 28 | 3.9 | 0.07 | 0.6 | 12 | 2.1 | 3.0 | 208 | 125 | 13 |
| | n | 5 | 5 | 4 | 4 | 9 | 9 | 9 | 5 | 9 | 9 | 6 |
| Fatty liver | $\bar{x}$ | 320 | 137 | 48.7 | 0.55 | 22.6** | 152 | 52.7** | 59.7** | 4326** | 1946* | 59.4* |
| Non fatty part | S.E.M. | 43 | 52 | 11.1 | 0.04 | 2.6 | 9 | 8.0 | 5.2 | 165 | 103 | 6.8 |
| | n | 4 | 4 | 4 | 4 | 7 | 8 | 8 | 5 | 8 | 7 | 8 |
| Fatty liver | $\bar{x}$ | 289 | 113 | 53.3* | 0.51 | 16.2** | 146 | 27.0** | 44.0* | 3682 | 1523 | 71.8 |
| Fatty part | S.E.M. | 10 | 19 | 5.5 | 0.07 | 1.7 | 19 | 5.0 | 2.5 | 297 | 179 | 15.3 |
| | n | 4 | 4 | 4 | 4 | 8 | 8 | 8 | 5 | 8 | 8 | 8 |

*) Significantly different from control $P < 0.05$
**) Significantly different from control $P < 0.01$

## Discussion

The present study revealed some biochemical changes in the liver and blood rats during liver fattening. Liver enzymes showing the largest changes were malic enzyme and glucose 6-phosphate dehydrogenase. The increased levels of these enzymes, which generate NADPH, a substrate required for the synthesis of fatty acids, suggest that excessive energy-rich diets lead to the induction of these lipogenic enzymes and that at least part of the lipids accumulated in the liver originated from de-novo synthesis of lipids. Although not to the same extent; levels of other enzymes were also elevated in the fatty liver, indicating increased metabolic activity.

A similar picture was seen in the fattened liver of the force-fed geese. Enzymes responsible for the generation of NADPH were significantly increased during liver fattening (5, 6). Furthermore, good correlations between the degrees of liver fattening and the levels of the lipogenic enzymes were seen.

The fattened liver was significantly enlarged, but there was relatively small damage to the hepatocytes, as evaluated by the mild change in the levels of the blood enzymes of hepatic origin. While the mean level of serum alanine aminotransferase increased by 68.7%, the level of serum aspartate aminotransferase increased only by 18.7%. The possible explanation for the large difference seen in the changes of these two closely related enzymes could stem from their location in the hepatocyte. While aspartate aminotransferase is mainly a mitochondrial enzyme and is found to a lesser extent in the cytoplasm, alanine aminotransferase is completely localized in the cytoplasm. Therefore, during mild cellular damage, only the outside cellular membrane is damaged, without affecting the mitochondrial membrane, consequently leading to the leakage of the soluble cytoplasmic enzymes only.

The liver enzymes studied could be divided into three groups according to changes in their levels during liver fattening: increased, and decreased and unchanged. Dividing the fatty liver to fatty and non-fatty parts showed that the largest changes seen were in the non-fatty part of the fatty liver, while the values (except for aldolase), in the fatty part were between those of the non-fatty part and the controls. This phenomenon may suggest that prior to fat accumulation in the hepatocytes, enzyme induction takes place, reaching a peak and then declining after fat accumulation occurs. It is possible that this process is paralleled by two signals, first by the energy rich diet leading to enzyme induction, followed by the second

signal to stop this effect after excessive fat accumulation in the liver which leads to damages of its functions.

It is of interest that those enzymes showing increased activities at the early stage of lipid accumulation decreased whenever the cells started to accumulate fat and, vice versa, those declining at the early stage reversed the pattern and showed an increased level in comparison to the prefattened stage.

The liver and the adipose tissue are in equilibrium. Whenever an excessive high energy rich diet is given, adaptation of the liver to increased lipogenesis and eventual transport of fat to the adipocytes occurs. At this stage, the liver enzyme pattern changed, showing the enzyme pattern seen in the non-fatty part of the fatty liver. Prolonged exposure to these conditions, eventually leads to fat accumulation in the liver and its fattening, at which stage a reversed process takes place, showing an enzyme pattern seen in the fatty part of the fatty liver. This process is not clear, but it could result from either a process of self protection by lowering lipogenesis capacity or a start of liver degeneration.

The fact that liver size increased and protein content decreased by similar values, clearly indicates that the change was due to an increase in the lipid content of the hepatocytes, leading to the dilution of the proteins in the fatty liver. Whenever enzyme activities were calculated on the basis of mg protein, the dilution of the enzyme in the hepatocyte did not affect the specific activity of the enzyme. This however would give different values if activities were determined on a fresh weight basis.

The causes of lipids accumulation in the hepatocytes are not clear. The transport of lipids to the depots and vice versa is of considerable metabolic importance. In a case of caloric balance, a considerable fraction of the depot liquid is turned over, with significant amounts mobilized to the various tissues. On the basis of the increase of total lipids in the blood, it seems that there is no strong lipaemia following the massive fat mobilization from the fat depots which may lead to fatty liver. It seems therefore that liver fattening in this experimental model resulted from some metabolic imbalances. It is possible that lipid accumulation in the liver resulted from lack of very low density lipoproteins, which have a key rôle in lipid mobilization from the liver. It was shown that orotic acid in the diet of rats leads to the reduction of blood apolipoprotein B, a component of the very low density lipoproteins (7). Additional information may be gained from data obtained from another model of the rat, in which fatty liver was induced by a choline-deficient diet. It was pistulated that due to lack of choline, the phosphoglyceride, phosphatidylcholine was also deficient. This, in turn, decreases one of the main routes of lipid export from the liver, resulting in its accumulation in the liver and causing fatty liver (12, 13). In this model, it was also reported that the levels of liver enzymes aspartate aminotransferase, alanine aminotransferase and glutamic dehydrogenase increased significantly in parallel to liver fattening. This hypothesis is confirmed by our studies (unpublished) with cattle fatty liver, which contains decreased levels of phospholipids.

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